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CHARACTERISTICS OF THE MICROBIOM OF THE NECK REGION OF THE TEETH IN THE EXPERIMENTAL ACTION OF OPIOID, ITS CANCELLATION AND APPLICATION OF CEFTRIAZONE AS A COMPOSITION OF COMPLEX MEDICAL CORRECTION

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ABSTRACT

The aim of the study was to determine the changes in the qualitative and quantitative composition of the microbiota in the neck part of the teeth in the gingival margin of the oral cavity of rats under experimental opioid exposure, its cancellation and comprehensive drug correction using an antibacterial drug. The use of the antibiotic ceftriaxone under conditions of two- and six-week administration of the opioid analgesic nalbuphine, caused the elimination or reduction of the quantitative composition of opportunistic and pathogenic bacterial species, in contrast to the identified changes in the microbiocenosis in the studied microbiotope. antibiotic therapy in the experiment.

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The results of the article correspond to the research plan of Lviv National Medical University named after Danylo Halytsky and are part of the research topic of the Department of Normal Anatomy "Morpho-functional features of organs in the pre- and postnatal periods of ontogenesis, under the influence of opioids, food additives, reconstructive surgery and obesity", state registration number 0120U002129.

In pain syndrome in clinical practice, an important component is the appointment of opioid drugs [12]. Long-term use of opioids often begins with the treatment of acute pain, however, to date there are insufficient data to characterize the transition from acute to chronic opioid use [13]. Prolonged abuse of opioids leads to various complications with systemic organ damage. Among the pathology of the oral cavity, periodontal tissue lesions occupy one of the leading places, because these tissues reflect the influence of harmful factors and homeostasis, and the mucous membrane is one of the first target organs that responds to intoxication by harmful factors [1, 5, 10, 11]. In opioid-dependent individuals, the heterogeneity of the microbiocenosis prevails in both qualitative and quantitative composition of the bacterial flora, which also depends on the duration of drug abuse [2, 6]. Since one of the main etiological factors is recognized as microbial, there is a need to focus on the

basic principles of therapy primarily to eliminate plaque and dental plaque, which cause the development of inflammation in the oral cavity [3, 4, 8, 14]. Due to the great variety of mechanisms of colonization and virulence of pathogenic microorganisms of dental plaque, the use of local and systemic antibiotics is extremely necessary [8]. An important aspect for the selection of effective etiotropic treatment is testing the degree of sensitivity of bacterial isolates to antibiotics [7, 9]. However, in the available sources we did not find data on the peculiarities of microbiome changes in the cervical part of the teeth and gingival margin at different stages of opioid exposure and, accordingly, etiopathogenetic treatment with antibacterial drugs under such conditions.

The aim of the study was to determine the changes in the qualitative and quantitative composition of the microbiome in the cervical part of the teeth in the gingival margin of the oral cavity of rats under experimental opioid exposure, its cancellation and complex drug correction using a course of antibiotic therapy with ceftriaxone.

Materials and methods. The study material was white outbred male rats (58), average body weight 180 g, age 4.5 months. The duration of the experiment was six weeks. Experimental animals were divided into 5 groups. The first group consisted of 10 intact rats. Each experimental group included 12 individuals. In the second group, the opioid analgesic nalbuphine was administered daily intramuscularly for six weeks, and the average therapeutic dose for rats was 0.212 to 0.252 mg / kg. The third group - rats were injected with nalbuphine - 0.212 mg / kg during the first two weeks, followed by its cancellation. In the fourth group, the animals were administered an opioid analgesic at a dose of 0.212 mg / kg for two weeks, followed by a four-week drug withdrawal and drug correction with pentoxifylline (4 weeks) and ceftriaxone (11 days) at doses of 2.86 mg / kg. The fifth group - rats for six weeks were injected with nalbuphine (from 0.212 to 0.252 mg / kg.), As well as pentoxifylline in the period from the third to the sixth week and 11 days of the antibiotic ceftriaxone (32-42 days) - 2, 86 mg / kg. The animals were kept in standard vivarium conditions and all experiments were performed in compliance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [Strasbourg, 1985].

Collection of microflora for bacterioscopic and bacteriological studies was performed in the cervical part of the teeth in the gingival margin of the oral cavity of rats. In the previous stage, it was found that the studied bacterial strains were more sensitive and sensitive to ceftriaxone compared to other antibacterial drugs. According to the instructions for medical use, the duration of therapy with ceftriaxone is from 4 to 14 days, however, in chronic infections, longer use is recommended, and in the presence of *Streptococcus pyogenes* - at least 10 days. In this case, the maximum allowable dose of antibiotic - up to 4 g per day. Therefore, we chose a regimen of ceftriaxone therapy - 11 days, at a dosage of 1 g per day. In terms of rats, the dose was 2.86 mg / kg. In bacterioscopic studies, the assessment of prokaryotic gram-positive and gram-negative microflora was performed on the quantitative relationships of the main elements in the smear. To identify the characteristic cultural characteristics and quantitative characteristics of the microflora, bacteriological studies were performed by performing cultures on standard nutrient media. The obtained data, for further statistical analysis, were tested for normality by calculating the coefficients of asymmetry and excess using the Shapiro-Wilk test (significance level $p < 0.05$). Because all data obtained were of a normal distribution, the central trend was presented as $M \pm SD$ (mean \pm standard deviation). All statistical calculations were performed using RStudio v. 1.1.442 and R Commander v.2.4-4.

Research results. In the third group of animals, the analysis of the species and quantitative composition of the microbiota of the studied subbiotope of the oral cavity of rats was performed at the end of the sixth week of the experiment. The study of microbiota features in the studied subbiotope of the oral cavity of rats showed changes in the aerobic microflora, especially in the quantitative characteristics of individual species. According to the results of a statistical study, the significance of the difference for all microbial groups at different terms of opioid exposure during the experiment was proved ($p < 0.0001$).

Bacterioscopic examinations revealed mainly gram-positive microflora in the form of rod-shaped or filamentous bacteria, individual clusters of coccal flora. Gram-negative rods and yeast-like cells were found singly in the smears. Neutrophilic leukocytes, degeneratively altered and destroyed epitheliocytes, as well as epithelial cells with adsorbed coccal microflora up to 15 in the field of view were visualized.

In bacteriological studies, it was found that the state of the microbiocenosis in the studied subbiotope of the oral cavity varied compared with the intact group of rats, as well as with group 2 animals treated with nalbuphine for six weeks. Quantitative indicators of normal microflora in group 3

increased in comparison with similar indicators of intact rats. Thus, the number of non-hemolytic streptococci increased 1.3 times compared to the intact group - up to 60.78 ± 6.72 CFU / 0.02 ml. and gram-positive non-spore rods 1.7 times - 15.22 ± 4.99 CFU / 0.02 ml. At the same time, there was no significant difference with the corresponding indicators in animals of group 2, which were administered opioids for six weeks. The number of gram-positive spores was 13.22 ± 2.17 CFU / 0.02 ml, which did not differ significantly from similar intact animals and decreased 1.4-fold compared to animals treated with opioids for six weeks.

Quantitative composition of opportunistic pathogenic microflora was characterized by different indicators. Quantitative indicators of α -hemolytic streptococci in group 3 did not differ from the corresponding indicators of intact animals and amounted to 65.33 ± 7.57 CFU / 0.02 ml., However, were 1.6 times higher than similar indicators of group 2 animals. There was a significant increase in the number of coagulase-negative staphylococci to 61.56 ± 4.07 CFU / 0.02 ml, which was 5.9 times more than in the intact group of rats and 1.7 times compared with the corresponding indicators in animals with opioid administration for six weeks (2 groups). The number of enterococci did not differ from the intact group of rats, which was at this time - 19.22 ± 5.24 CFU / 0.02 ml and decreased 1.6 times compared with animals with six weeks of opioid administration. A significant decrease in the quantitative indicators of enterobacteria was recorded. Thus, the number of *Escherichia coli* colonies was 4.00 ± 0.71 CFU / 0.02 ml, which was 1.7 times less than in intact animals and 7.0 times compared to similar indicators in group 2.

At the same time, a small number of *Escherichia coli* colonies was noted - 3.11 ± 0.93 CFU / 0.02 ml, which was 6.8 times less than in the 2nd group of animals.

However, these enterobacteria, which show signs of pathogenicity, were absent in intact rats. Attention was drawn to the appearance of *Staphylococcus aureus* - a microbial group of coagulase-positive staphylococci - in the amount of 6.00 ± 0.71 CFU / 0.02 ml. This species was not sown in intact animals, and in the group of animals exposed to six weeks of analgesics, their number was greater. Fungal microflora in the amount of 6.00 ± 0.50 CFU / 0.02 ml and single colonies (1-2) of *Klebsiella* were sown on standard nutrient media. Colonies of beta-hemolytic streptococci, bacteroids, and *Pseudomonas aeruginosa* were not sown in this subgroup (Table 1).

Table 1. Species and quantitative composition of the microflora of the cervical part of the teeth and gingival margin of rats at the end of the sixth week of the experiment, under conditions of opioid withdrawal and drug correction (CFU / 0.02 ml).

№	Bacterial groups	Intact group	After 6 weeks of opioid action	2 weeks - opioid, 4 weeks - cancellation	2 weeks - opioid, 4 weeks - cancellation and correction	6 weeks - opioid, 4 weeks - correction
1.	Non-hemolytic streptococci	45.11±5.16	60.67±5.55	60.78±6.72	4.00±0.71	16.11±2.03
2.	Gram-positive non-spore rods	9.00±1.22	18.00±2.45	15.22±4.99	-	-
3.	Gram-positive spores rods	9.00±1.12	19.00±2.65	13.22±2.17	-	-
4.	α -hemolytic streptococci	65.44±4.36	40.56±6.02	65.33±7.57	9.11±1.05	7.00±0.71
5.	Coagulase-negative staphylococci	10.33±3.67	35.78±4.66	61.56±4.07	4.00±0.87	14.11±1.54
6.	Enterococci	18.33±2.96	30.22±6.40	19.22±5.24	21.33±5.85	14.22±1.92
7.	<i>Escherichia coli</i>	7.00±1.50	28.11±2.71	4.00±0.71	9.22±2.22	10.11±2.09
8.	Hemolytic <i>Escherichia coli</i>	-	20.33±5.00	3.11±0.93	-	-
9.	β -hemolytic streptococci	-	27.11±5.93	-	-	-
10	Coagulase Positive staphylococci	-	16.11±2.03	6.00±0.71	-	-
11	<i>Klebsiella</i>	-	12.00±1.50	1-2	-	-
12	Fungal microflora	-	1-2	6.00±0.50	16.33±2.29	14.22±3.07

According to the experimental scheme, animals of group 4 were exposed to opioid for two weeks, followed by withdrawal and drug correction for four weeks, using an 11-day course of ceftriaxone at the end of the experiment. Bacterioscopic smears mainly visualized gram-positive microflora in the form of small clusters or short chains of coccal microorganisms, as well as a small number of epithelial cells with cocci adsorbed on their surfaces. Obligatory anaerobic gram-negative bacteroids were not detected in the field of view.

Bacteriological studies after a course of antibiotic therapy revealed a decrease in the quantitative composition of non-hemolytic streptococci in 11 times compared with intact animals - up to 4.00 ± 0.71 CFU / 002 ml. and, accordingly, 15 times compared to similar animals when opioid was administered for six weeks. Gram-positive non-spore and gram-positive spore bands naturally sensitive to antibiotics were not detected.

Quantitative indicators of opportunistic bacterial species also decreased. Thus, the number of α -hemolytic streptococci decreased 7.2 times compared with the intact group of rats - up to 9.11 ± 1.05 CFU / 002 ml and 4.5 times compared with six weeks of opioid administration. The number of coagulase-negative staphylococci was halved to 4.00 ± 0.87 CFU / 002 ml compared to intact animals and 8.9 times compared to similar animals in opioid administration for six weeks. Significant differences in the quantitative indicators of enterococci (21.33 ± 5.85 CFU / 002 ml.) Compared with the intact group of animals were not detected, however, their number decreased by 1.4 times, compared with the corresponding indicators in animals with six weeks of opioid action. During this period of the study, the quantitative indicators of *Escherichia coli* (9.22 ± 2.22 CFU / 002 ml.) Remained close to the indicators of the intact group of animals, however, decreased by 3.1 times compared with the corresponding indicators in animals with opioids for six weeks. In animals of this subgroup were also sown *Candida albicans* fungal microflora in the amount of 16.33 ± 2.29 CFU / 002 ml. It should be noted that pathogenic bacterial species, such as β -hemolytic streptococci and coagulase-positive staphylococci were eliminated as a result of experimental antibiotic therapy (table 1).

In group 5 animals, in order to study the effectiveness of pathogenetic treatment of cervical and gingival dysbiosis, which developed with prolonged opioid exposure for six weeks, antibacterial therapy with ceftriaxone was used as part of a comprehensive drug correction 32 days at the end of the experiment. Bacterioscopic studies of smears in the study area indicated a decrease in the activity of formation of extracellular structures in the form of a biofilm, the predominance of gram-positive microflora and epitheliocytes with adsorbed coccal microflora.

Bacteriological studies in group 5 of animals showed changes in the species and quantitative composition of the microbiota of this subbiotope of the oral cavity. In particular, the quantitative values of non-hemolytic streptococci decreased 2.8 times - up to 16.11 ± 2.03 CFU / 002 ml compared to the intact group of animals and 3.7 times compared to the group in animals treated with opioids for six weeks without corrective action. Gram-positive non-spore and spore rods that were naturally sensitive to antibiotics were not sown on standard nutrient media (Table 1). The quantitative composition of opportunistic bacterial species also changed significantly. Thus, the number of α -hemolytic streptococci decreased 9.3 times - up to 7.00 ± 0.71 CFU / 002 ml compared with the intact group of animals and 5.8 times compared with those in animals with six weeks of opioid administration without antibiotic therapy. There was a slight increase in the quantitative indicators of coagulase-negative staphylococci - up to 14.11 ± 1.54 CFU / 0.02 ml. compared with intact animals, however, the rates decreased 2.5-fold compared with opioid-treated animals for six weeks without corrective action.

The number of enterococci (14.22 ± 1.92 CFU / 002 ml.) Was slightly reduced compared to the corresponding values in intact animals, and was twice lower than in animals with six weeks of opioid administration without drug correction. The quantitative composition of *Escherichia coli* was 10.11 ± 2.09 CFU / 002 ml, ie did not differ significantly from intact animals, but decreased by 2.8 times compared to animals treated with opioids for six weeks without the use of antibacterial drugs. Under the action of ceftriaxone, hemolytic *Escherichia coli* was eliminated at the sixth week, but the fungal microflora remained in the amount of 14.22 ± 3.07 CFU / 002 ml. In almost all cases, under the conditions of antibiotic administration, at the end of the experiment there were signs of significant restriction of pathogenic bacterial groups, in particular, no β -hemolytic streptococci and coagulase-positive staphylococci were sown (Table 1).

Conclusions. 1. Detected changes in the microbiome under the action of opioids for two weeks and its four-week withdrawal indicated a violation of microbiocenoses in the neck region in the gingival margin and as a consequence - a probable violation of protective antimicrobial factors of the oral cavity of animals.

2. In animals exposed to nalbuphine for two weeks, followed by discontinuation and administration of ceftriaxone antibacterial therapy, there were signs of elimination or reduction in the quantitative composition of bacterial species that are potentially etiological agents of inflammatory processes.

3. With the introduction of ceftriaxone on the background of opioid activity for six weeks eliminated bacterial species that are likely to cause inflammation, and the number of other indicator species decreased, indicating the normalization of microbiocenoses of the studied biotope compared with animals correction under such conditions.

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